The Somatic Mitosis of Stegomyia fasciata.

By

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With Plate 21.

Introduction and Material.

In the summer of 1917 Dr. Ashworth sent some eggs of Stegomyia fasciata to Prof. Graham Kerr, of Glasgow University, for museum purposes. These eggs had been obtained from females of a strain brought from Freetown, West Africa, by Mr. A. Bacot, F.E.S., Entomologist to the Lister Institute, London, successfully bred by him in London since his return.

Some of the eggs were incubated in the Laboratory of the College of Notre Dame where Sister Monica Taylor (D.Sc.) found their development to be unusually rapid, the fulfilment of a condition necessary for the successful study of somatic mitosis, in order that a copious supply of dividing nuclei may be secured in a single specimen—thus rendering the seriation of stages possible.

While awaiting the discovery of a technique which will overcome the difficulties of sectioning later stages of the eggs of Culex pipiens, in order to furnish material for tracing out the gradual evolution of parasyndetic conditions in Culex, she had suggested that I should investigate the somatic mitosis in another mosquito.

The arrival of the rapidly developing Stegomyia

fasciata furnished material admirably suited for the proposed study.

Prof. Graham Kerr gave up his surplus supply and Dr. Ashworth very courteously added to his original gift by sending fresh batches direct to the College. I take this opportunity of thanking the donors for their kindness and liberality, and also record my thanks to Sister Monica for her advice and helpful criticism throughout.

This short investigation has, therefore, been made with a view to determining whether the pairing of the Chromosomes in the somatic tissues exists, and, if so, of possibly linking up by means of another species, with a less pronounced condition of parasyndesis, the egg of Culex pipiens with the later stages of development.

Very much work remains to be done on the passage of the telophase nucleus into the resting nucleus, and since in C. pipiens the telophases of the later spermatogonial divisions were observed to pass directly into the synizetic nuclei, it seemed probable, as already mentioned, that mosquito material would be favourable for such a study, as it has so proved.

Abundant cases of dividing nuclei were found in the body-wall cells, particularly in larvæ just about to undergo ecdysis. Tracheal tube cells, as well as cells of other tissues also showed occasionally many cases.

The methods used were the same as those employed for C. pipiens, i.e. small portions of the mosquito were fixed in the fluids known as Flemming, Gilson-Petrunkewitsch, in warm solution of corrosive sublimate in alcohol. (The fixative used for any particular study is indicated in the explanation of plates.) Sagittal sections 8μ thick were used exclusively, and all nuclei described and figured were wholly within one section. This precaution is necessary where the chromosomes are long as in this case.

The Flemming fixation has the effect of making the nuclei look larger. It does not, however, so clearly preserve the spindle apparatus, which appears to greater advantage in

other fixatives where, at the same time, the chromosomes also stand out just as sharply defined and are as readily counted as in the Flemming. The stain was Heidenhain's hæmatoxylin throughout.

The gonad having the same topographical relations as in Culex, the third segment from the hind end of the abdomen was cut out and sectioned in order that the spermatogonial and oogonial mitosis might be investigated.

Both the ovary and testis are similar in structure to those of Culex pipiens, and need not be described in detail. The testis is divided up into a number of compartments, all the cells of one compartment being approximately at the same stage of division. As might be expected in the more rapidly developing species, a greater range of "spermatogenesis" stages is obtainable in one specimen—large early spermatogonia, smaller spermatogonia of the multiplication zone, primary and secondary spermatocytes, and complete spermatozoa often being visible in a single section.

The later stages of spermatogenesis follow quite closely those described for C. pipiens (**3**) (Pl. 21, fig. 29), and throw no special light on parasyndesis, hence they have not been included in this account.

Most probably because of its rapid development—the imago in some cases emerging from the pupal case seven days after the larva hatched—the synizetic nucleus which occurs so abundantly in the C. pipiens testis is not nearly so plentiful in Stegomyia, although, as will be shown later, the synizetic stages are analogous in the two mosquitoes. Thus the assumption that the synizetic nucleus of the male gonad functions as a sort of inactive phase in C. pipiens seems justified. Later it will be shown that the so-called synizetic nucleus in Stegomyia is really the stage characteristic of the nuclei of the two newly-formed daughter-cells, i.e. the stage following immediately on the telophase.

A synizetic-like stage in somatic—apart from spermatogonial and oogonial—mitosis was noticed in C. pipiens, but its connection with the telophase of a dividing nucleus could

not be demonstrated. The ovary in the larva and pupa of Stegomyia is quite immature. It apparently grows rapidly and attains its full development when the imago has been suitably fed. Synizetic-like nuclei are much more numerous in the tissues of the immature ovary than in those of the testis. In the former the growth is relatively slower, and may be suspended altogether until the suitable diet has been supplied to the imago. Hence here the synizetic-like nucleus seems to function as a "waiting" nucleus.

There is no difficulty in determining four as the diploid number of chromosomes.

Somatic Mitosis. (Passage of the telophase nucleus into the resting nucleus.)

I. Synizesis. (Pl. 21, figs. 1, 2, 3*a*.)

As already indicated, the two masses of chromatin formed from four daughter-chromosomes which cap the pole of the spindle in late telophase become surrounded by a clear liquid as the nuclear membrane is completed round the daughter-products.

(N.B.—The nuclear membrane persists during mitosis as in C. pipiens.)

The spindle-fibres either disappear altogether or are drawn into the chromatin masses and the synizetic nucleus is to be seen in each of the two daughter-cells. These two nuclei become gradually separated one from the other by the growth of the cytoplasm surrounding them, but they can easily be detected until they have almost reached the resting condition. This account holds good for all the fixatives employed.

II. Synizesis (i.e. late Telophase) to Resting Stage (Pl. 21, figs. 3–6).

The telophasic mass becomes gradually invaded by the surrounding nuclear sap, and assumes a vacuolated appearance.

Strands of pale staining substance are now seen to connect the chromosomic mass with the nuclear membrane, and there is a tendency for the chromatin to be drawn towards the membrane.

In favourable specimens at this stage a condition similar in form to that of an early prophase can be detected (Pl. 21, figs. 3c and 4)—i.e. the chromatin has the appearance of two pairs of chromosomes—the members of the pairs being very much twisted one around the other. A comparison of Pl. 21, fig. 17 (an early prophase in a young testis where the number of compartments making up the whole gonad is twelve) with Pl. 21, figs. 3c and 4 brings out the likeness between a very late telophase and an early prophase, these interesting figures (3c and 4) illustrating undoubted cases of the telophase. There is, however, a difference in the staining capacity of the two stages, the early prophase showing a freshness and limpidity which is absent in the later stage of telophase.

Pegs of chromatin now begin to extend from the chromosomes, to lengthen and to anastomose, and as the chromosomes become more vacuolated the nucleus presents the appearance of a crown (Pl. 21, fig. 5), the nucleolus being conspicuous. Eventually the chromatin is fairly well distributed in blotches and patches, and a typical resting nucleus is the result (Pl. 21, fig. 6).

From the above description it would seem that there is a marked tendency for the chromosomes to emerge from the telophasic mass in two pairs, the outline of these two pairs being gradually obscured as the vacuolisation of the chromatin proceeds.

III. Resting Stage to Prophase.

At the outset of mitosis the chromatin assumes the character of a densely matted mass of very fine threads—difficult to stain—surrounding a characteristic deeply stained nucleolus (Pl. 21, fig. 7). In fact, this latter structure often looks like an irregular blob of chromatin; and since it gradually disappears as the new chromosomes become recognisable, it

would seem as though it had acted as a storehouse of chromatin ready to furnish the prophase chromosomes.

The incipient chromosomes now begin to thicken up in places and to contract. Liquid collects in the nucleus, which becomes more distended in consequence, and this liquid apparently pushes out the newly-forming chromosomes towards the nuclear membrane (Pl. 21, fig. 8). In this, the early prophase condition, two very long chromosomes can be distinguished (Pl. 21, fig. 9). They are double, the two membranes of the pair being twisted about each other (Pl. 21, fig. 9). Gradually a condensation proceeds, the pairs untwist, but the degree of untwisting varies greatly. When the untwisting has been slight—i. e. when the full condensation of the chromosomes has been effected before the component chromosomes of the pair have succeeded in extricating themselves from their close approximation—then, in full prophase, there are apparently only two chromosomes (Pl. 21, figs. 11, 12, 13.)

When the untwisting process is completed simultaneously with the full condensation of the chromosomes, then the members of the pairs lie side by side (Pl. 21, fig. 14). Sometimes the untwisting is completed before full condensation, when four chromosomes showing only slight parasyndesis are the result (Pl. 21, figs. 15, 16).

That faulty fixation has nothing to do with the varying appearance of two or four chromosomes is evident from the fact that the same phenomena occur whatever the fixatives employed, and in all the tissues—parasyndesis not being confined to any one or other of the somatic tissues.

There is no method of distinguishing the spermatogonial prophase from that of the primary spermatocyte. The degree of parasyndesis is always great in the gonad cells—the fully condensed chromosome always giving the impression of the haploid number. Studies made from the youngest compartment in young gonads composed of few compartments show a meiotic-like character of chromosome like that occurring in meiosis ring, S-shaped and 8-shaped figures being common (Pl. 21, figs. 17, 18).

These appearances in prophase are, however, followed by an anaphase which is typically somatic, and the nuclei can, therefore, be distinguished as spermatogonial in anaphase (Pl. 21, fig. 28). As in C. pipiens, the primary spermatocyte cells are spindle-shaped, whole chromosomes being separated in the first meiotic division (Pl. 21, fig. 29).

IV. Metaphase and Anaphase.

The two pairs of long chromosomes arrange themselves towards the centre of the nucleus, parallel to the plane of the future equator of the spindle (Pl. 21, fig. 19).

In the large cells, where the chromosomes are very long, i.e. longer than the equator, they accommodate themselves by twisting up in a zig-zag fashion. As a consequence of this they are often difficult to count. In favourable specimens of small cells, however, they can be distinguished, and the longditudinal splitting can be observed. Most probably because of this great length the usual V-shaped chromosome of anaphase is often replaced in Stegomyia by a W-shape (Pl. 21, fig. 25).

That there is a tendency for the homologous chromosomes to become clearly associated in their passage to the poles can be demonstrated in many cases of uncut nuclei where this association can in no wise be attributed to bad fixation. As a result, only two chromosomes can in such cases be detected at the poles (Pl. 21, figs. 23, 24, 27).

In other cases, however, the four chromosomes at the poles can be counted quite readily (Pl. 21, fig. 26), and that the members of homologous pairs are sometimes separated is evident from a study of the sizes of the chromosomes (Pl. 21, fig. 25).

As explained above, the long chromosome has a W-shape in anaphase, the short chromosome either a or rod-shape. This alternate arrangement of W, with a rod at each pole of the spindle, must betoken a separation of the members of the pairs. In cases such as Pl. 21, fig. 25, the repairing of

the homologous chromosomes would appear to take place in the synizetic mass, since as explained above, two pairs of chromosomes can often be detected in late telophase.

From the foregoing observations it will be seen that Stegomyia fasciata offers no exception to the parasyndetic condition, as worked out by Metz (**2**), for other Diptera. In fact, the varying degrees in which that condition exists in this mosquito may be considered to place Stegomyia in a median position, acting as a link between the extremes of little or no parasyndesis in the segmenting-egg stage of Culex pipiens, and the complete parasyndesis as found in the later stages of development of the same animal.

Summary.

(1) The diploid number of chromosomes in Stegomyia fasciata is four.

(2) A varying degree of parasyndesis (pairing of the chromosomes) is exhibited in the somatic cells, extreme parasyndesis giving a haploid count.

(3) Each telophasic mass of chromatin gives rise directly to a synizetic nucleus instead of the usual "resting" nucleus.

(4) The nuclear membrane persists throughout mitosis.

(5) The homologous chromosomes pair either in anaphase or in telophase.

Note.—At the Editor's request I append the following short list of certain terms used in this paper, together with the name of the author of each and the publication in which it appeared.

Anaphase (G. ana, back or again), introduced by Strasburger, 1884, in his paper, "Die Controversen der indirecten Kerntheilung" ('Arch. für Micr. Anat.,' xxiii, p. 260). His own definition translated runs thus: "The phases passed through (by the nucleus) from the complete separation of the daughter-segments to the final establishment of the daughter-nuclei may be known as the anaphases of the division," i.e. the phases during which the nucleus returns to its original condition.

As used by Strasburger, it will be seen that his term "Anaphasen" includes the more modern "telophase." The restricted definition of anaphase is now "the later period of mitosis during which the divergence of the chromosomes takes place," i. e. the passage of the chromosomes from the equatorial plate to the poles, when the telophases now begin.

Diploid (G. diploos, double), as opposed to haploid, q. v.

It is employed to indicate the normal number of chromosomes present in the nucleus of a somatic cell (Strasburger, 1906).

Haploid (G. haploos, single or simple), indicating the number of chromosomes present in the nucleus at the end of the "reduction" period, that is, the number in each ultimate germ-cell. On the conjugation of the male and female germ-nuclei a nucleus is produced containing double this "haploid" number, or what is known as the "diploid" number of chromosomes.

Both words haploid and diploid were introduced by Strasburger in his paper, "Typische und allotypische Kernteilung" ('Jahr. f. wiss. Bot.,' vol. xlii, p. 62, 1906), thus:

"Finally, it is desirable that the terms Gametophyte and Sporophyte, at present confined to plants with the single and double chromosome number respectively, should be discarded when the distinction is extended to the animal kingdom. To this end I propose the words Haploid and Diploid, or Haploid and Diploid generation."

Meiosis (G. meiosis, lessening), first used by J. B. Farmer and J. E. S. Moore in 1905 in their paper, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants" ('Quart. Journ. Micr. Sci.,' vol. 48, N.S.).

It is the period during which the reduction in the number of chromosomes takes place, including both maturation divisions.

Metaphase (G. meta, between), introduced by Strasburger with Prophase and Anaphase, q. v., the "middle stage" of the mitosis. He says: "The stages from the beginning

of the breaking asunder of the daughter-segments to their complete separation and arrangement in a ring (round the spindle axis) I would term the Metaphases."

This definition still holds good. It will be noted that Strasburger employs these terms Anaphase, etc., in the plural —Anaphasen, Metaphasen, etc.—thus indicating a series of changes in each case.

Parasyndesis (para, beside). The longitudinal pairing of the chromosomes—as opposed to Metasyndesis—introduced by Häcker, 1909, in "Die Chromosomen als Angenommene Vererbungsträger" ('Ergeb. u. Fortsch. der Zool.,' vol. i, p. 74). Thus: "For the pairing of the chromosomes I would, as already mentioned, keep the term Syndesis. When, in order to effect this union, the chromosomes arrange themselves (in pairs) with their long axes parallel, I propose to call this arrangement a Parasyndese (Paradese), or paradetic union. When the arrangement of the chromosomes is "end to end," the term metasyndese (metadese) or metadetic coupling would be more appropriate.

Prophase (pro, first). Introduced by Strasburger with Anaphase, q. v., thus: "The introductory phases of the nuclear division which I shall call, collectively, Prophases, begin with the appearance of the filament-skein (spireme). . . . Finally, the nuclear plate is ready to form, and herewith the prophases of the division terminate."

Resting Nucleus.—A nucleus in a state of vegetative activity, in which the chromatin is not arranged in the form of compact chromosomes; a nucleus "nesting" from actual mitosis—the period between two successive nuclear divisions.

Synizesis (G. syn, with; hizo, place), introduced by McClung, 1905, in "The Chromosome Complex of Orthopteran Spermatocytes" ('Biol. Bull.,' ix, 1905).

It indicates the clumping together of the chromatin often observed in the meiotic prophase. It was included in Moore's earlier term, "synapsis."

Telophases (telos, end), first used by Heidenhain, 1894 in the "Neue Untersuchungen über die Centralkörper und ihre Beziehungen zum Kern und Zellenprotoplasma" ('A. m. A.,' xliii).

The closing phases of mitosis, from the massing together of the chromosomes at the two poles of the nuclear spindle up to the final establishment of the daughter-nuclei—i. e. to the end of the mitosis.

Literature.

1. Metz, C. W. (1914).—"Chromosome Studies in the Diptera. I. A Preliminary Survey of Five Different Types of Chromosome Groups in the Genus Drosophila." 'Journ. Exper. Zool..' xvii.
2. ——— (1916).—"Chromosome Studies in the Diptera. II. The Paired Association of Chromosomes in the Diptera and its Significance," ibid., xxi.
3. Taylor, Monica (1914).—"The Chromosome Complex of Culex pipiens." 'Quart. Journ. Micr. Sci.' (N.S.), vol. 60.
4. ——— (1917).—"The Chromosome Complex of Culex pipiens." ibid., (N.S.), vol. 62.

EXPLANATION OF PLATE 21,

Illustrating Miss Lucy A. Carter's paper on "The Somatic Mitosis of Stegomyia fasciata."

[All figures were drawn with the Abbe camera under Leitz' $\frac{1}{12}$ in. oil immersion objective and Zeiss' compensating ocular 12. Magnification of 3500 diameters. The magnification of the figures as reproduced is shown by scale. Fixatives indicated: Gilson Petrunkewitsch, G.P. Flemming, F. Corrosive sublimate in alcohol (warm), C.A. All sections stained with Heidenhain's hæmatoxylin.]

Fig. 1.—Cell from gonad. Telophase passing into resting stage. G.P.

Fig. 2.—Nucleus passing into synizesis. Cell from body-wall. G.P.

Fig. 3.—Telophase—three stages *a*, *b*, *c*, from group of nerve cells. In *c*, two pairs of chromosomes coming out of the mass. G.P.

Fig. 4.—Interesting stage of somatic telophase, showing paired character of threads coming out of the telophasic-synizetic mass. Cell from the body-wall of fairly old larva. F.

Fig. 5.—Somatic. Synizetic mass unfolding; gradual conversion into resting nucleus; paired character of threads. F.

Fig. 6.—Two resting nuclei from body-wall. F.

Fig. 7.—Somatic. Very early prophase showing chromatin passing to chromosomes from nucleolus; from larva. C.A.

Fig. 8.—Early prophase from Spermatocyte 1. Imago ♂. C.A.

Fig. 9.—Gonad cell showing double character of chromosomes. G.P.

Fig. 10.—Early prophase. Somatic cell from tracheal tube. G.P.

Fig. 11.—Prophase; two chromosomes—from ovary. G.P.

Fig. 12.—Somatic from body-wall. ♀. F.

Fig. 13.—Early full prophase in somatic cell from body-wall; chromosomes long and lying in the nuclear membrane. F.

Fig. 14.—Prophase; four chromosomes in two groups; cell from body-wall of fairly old larva. F.

Fig. 15.—As fig. 14.

Fig. 16.—Late full prophase; somatic; four chromosomes. F.

Fig. 17.—Cell from youngest compartment in a gonad consisting of twelve compartments; probably spermatogonial. F.

Fig. 18.—As in fig. 17. ♂.

Fig. 19.—Very early metaphase. Two long and two short chromosomes nearing the future equator. F.

Fig. 20.—Metaphase; somatic ♂; gonad has twenty-five compartments. F.

Fig. 20*a*.—Somatic from larva. C.A.

Fig. 21.—Metaphase; spindle threads well differentiated; apparently four chromosomes; from gonad ♀. G.P.

Fig. 22.—Early metaphase. Four chromosomes splitting longitudinally from body-wall of pupa. G.P. Faintly stained.

Fig. 23.—Metaphase showing chromosomes in groups of two; somatic cell from body-wall. ♀. F.

Fig. 24.—From gonad ♀, looks like two chromosomes. G.P.

Fig. 25.—Telophase. Somatic from fairly old larva. F. Two long daughter-chromosomes separated by the two short chromosomes.

Fig. 26.—Telophase. Somatic from body-wall of larva. C.A.

Fig. 27.—Telophase. Two somatic cells from alimentary canal wall of larva. C.A. The two long chromosomes have reached the poles, the two short ones are still at the equator.

Fig. 28.—Spermatogonial telophase from gonad (fifteen to eighteen compartments) of larva. G.P.

Fig. 29.—First meiotic division from pupa. F.